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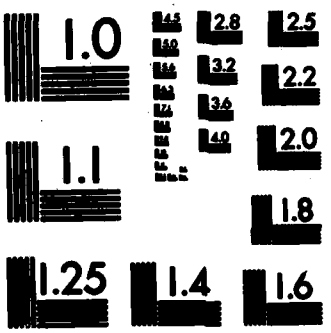

SALIVARY AND PLASMA TESTOSTERONE AND CORTISOL DURING MODERATELY HEAVY EXERCISE

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[illegible] of steroid hormones. High correlations between sali- [illegible] and 17-hydroxyprogesterone have been reported under [illegible] simple, noninvasive means of assessing steroid hormones. How- [illegible] of saliva to research topics relevant to the [illegible] comparability of salivary and plasma measures of [illegible]

[illegible] of their maximal oxygen consumption capacity. Paired [illegible] at the beginning of the run, at 15 minutes into the [illegible] for each sample by radioimmuno- [illegible] the saliva and plasma concentrations. [illegible] during the study.

[illegible] (r = .[illegible] to r = .[illegible]). [illegible] in either plasma or saliva due [illegible] during exercise for [illegible]

[illegible]

Introduction

The study of steroid hormones is of interest to exercise physiologists because these hormones influence metabolic substrate utilization and fluid and electrolyte balance. However, changes in levels of circulating steroid hormones during exercise have been inconsistent across studies (1-4). Resolution of these inconsistencies might be facilitated by more frequent sampling of the hormones to accurately quantify the temporal profile of hormone changes during exercise. Techniques that facilitate frequent sampling therefore may contribute to improved understanding of the physiological processes involved in exercise.

Standard blood collection procedures can be difficult to employ when frequently repeated measures are needed. Drawing blood for these measures can be troublesome due to problems arising from the need for repeated venipunctures. Catheterization provides an alternative, but is impractical in many settings. Venipuncture by either method may disturb the hormones to be measured (5).

Salivary measures provide a potentially sensitive alternative means of monitoring steroid hormones. Close parallels between salivary concentrations and plasma or serum concentrations have been demonstrated for cortisol(6-9), testosterone (10-13), 17-hydroxyprogesterone (14-16), and aldosterone (17). Salivary measures have the advantages of low risk, minimal stress, and ease of repetition during short time periods. However, this work has been done in clinical settings under resting conditions, and the findings may not generalize to conditions of interest to exercise physiologists. Thus, the present study was undertaken to examine the impact of a 30-minute period of moderately heavy exercise on the association between plasma and salivary measures of cortisol and testosterone.

Method

Sample

Fifteen male military and civilian personnel (12 white, three black) working for the U.S. Navy in San Diego, California, participated after giving informed consent and passing a medical screening examination. Additional descriptive data are given in Table 1. (See page 4.)

Exercise Protocol

The research design required participants to complete a 30-minute run at 75% of maximum oxygen consumption capacity (VO_2 max). Participants reported to the laboratory between 1300 and 1330 for VO_2 max assessment, which was measured in a single graded exercise session on a treadmill. Participants walked at 3.0 miles per hour (mph) at 0% grade for four minutes to warm up. The speed then was increased 0.5 mph each minute until a comfortable pace between 7 and 8 mph was reached. The grade then was increased 3% per minute until the participant voluntarily stopped running. Spirometry values during the run were determined by an online, open-circuit spirometry system[1] which recorded values on a magnetic tape and printed out 15-second averages. VO_2 max was reached when VO_2 did not increase in the one minute average during the test. The measured VO_2 max indicated that participants were above average in fitness (Table 1).

[1]The spirometry system consisted of an Applied Electrochemistry O_2 analyzer (Model S3-A), A Beckman CO_2 analyzer Model (LB-2), a KL Engineering pneumoscan spirometer (Model S-300), a Yellow Springs air temperature thermometer (Model 43T-A) and an Ewald Koegel one-way valve. All interfaced with a Hewlett-Packard Model 9825A programmable desk-top calculator.

TABLE 1
PARTICIPANT DESCRIPTION

	MEAN[a]	STANDARD DEVIATION
Age (years)	26.1	4.5
Height (cm)	177.2	8.5
Weight (kg)	76.6	10.4
Sum of skinfolds[b]	29.2	10.6
VO_2 max (l/min)	4.48	0.68
VO_2 (ml/kg min)	58.4	4.4
PHYSIOLOGICAL RESPONSES TO THE 30-MINUTE TREADMILL RUN		
Heart rate (bpm)	165.5	11.3
Ventilation (l/min BTPS)	92.8	18.8
VO_2 (l/min STPD)	3.49	0.50
VO_2 (ml/kg min STPD)	45.6	2.9
VO_2 (% VO_2 max)	77.5	4.2

[a] *N = 15.*
[b] *Computed from measures of biceps, triceps, suprailiac, and subscapular skinfold.*

The day following VO_2 max determination, the participants returned to the laboratory at the same time of day for the 30-minute run. Each participant ran at a speed calculated to represent 75% of his VO_2 max. On the average, actual VO_2 during the run was quite close to 75% (Table 1). This work level was expected to produce testosterone and cortisol changes in moderately conditioned individuals (1,2). Prior to beginning the run, the participants provided blood and saliva samples (see below) and then warmed up at six mph for five minutes. They then ran for 15 minutes, gave another blood and saliva sample, and completed the last 15 minutes of the run. At the end of the run, final blood and saliva samples were collected.

Blood and Saliva Collection and Assay Procedures

Venous blood was drawn for a superficial vein of the antecubital fossa into a 15-ml vacutainer collection tube. Samples were centrifuged immediately and the plasma separated from the red blood cells and frozen. Three-minute samples of whole saliva were collected by having the participants expectorate directly into 35-ml containers. Salivation was stimulated by chewing a 1.0 gm bolus of paraffin. Samples were frozen immediately after collection. The time required to collect both samples was three to four minutes, as blood was drawn during the saliva collection. In addition to the three samples taken during the 30-minute run, a baseline measure was taken the day before the run during the participants' initial laboratory visit prior to the VO_2 max determination.

To determine flow rate, saliva volume was estimated as follows: The vacutainer was first weighed empty. Empty weight was subtracted from the combined weight of the sample and the vacutainer to determine net sample weight, which then was translated to a volume estimate by assuming that the specific gravity of the saliva was 1.000. This assumption should have introduced an error of 1 percent at most into the volume estimates (18, pp. 22-23). Such an error is very small compared to the observed differences in estimated sample volume. Flow rate was finally determined as milliliters per minute by dividing the estimated volume by three.

The samples were stored frozen until assayed. All samples were measured in a single assay. Cortisol radioimmunoassay (RIA) employed the technique of Foster and Dunn (19) with intra-assay coefficients of variation of 17.6%, 5.2%, and 14.0% for low, medium, and high concentration pools, respectively. The testosterone RIA used the technique of Odell, et al. (20) with intra-assay coefficients of variation of 17.2%, 8.8%, and 2.0% for low, medium, and high concentration control pools, respectively. The RIAs measured total blood and total salivary hormone concentrations.

Analysis Procedures

Pearson product-moment correlations were used to describe the association between plasma and salivary concentrations for cortisol and testosterone. Additional analyses ensured that these product-moment correlations accurately summarized the data. The Kolmogoroff-Smirnov test (21) indicated that the score distributions met the assumption of normality. Nonparametric correlation (21) and jackknife statistics (22) showed that the parametric statistics yielded reasonable estimates of magnitude of association and significance, even if the normality assumptions had been relaxed. A multivariate analysis of variance for repeated measures was used to determine whether mean hormone levels changed significantly during the 30-minute run. All analyses except the jackknife statistics were performed with the Statistical Package for the Social Sciences (SPSS) (23). The jackknife statistics were computed on a hand calculator from correlations produced by the SPSS program.

Results

Cortisol levels did not change significantly during the study, but testosterone levels did (Table 2). Examination of values for individual participants showed that the nonsignificant cortisol trends represented the effect of averaging over people with very different response patterns. In the case of testosterone, there was a general correspondence in the trends for mean plasma and mean saliva hormone concentrations.

TABLE 2
PLASMA AND SALIVA CONCENTRATIONS OF CORTISOL AND TESTOSTERONE: MEAN LEVELS AND INTERCORRELATIONS

			BASELINE	PRE-EX	15 MINUTES	30 MINUTES	AVERAGE	F VALUE	SIGNIFICANCE
CORTISOL	Plasma	Mean[a]	84.40	94.93	91.87	95.27	91.62	2.17	.145
		S. D.	22.72	32.44	29.77	33.81	29.55		
	Saliva	Mean[a]	1.89	2.31	2.12	2.47	2.20	1.84	.194
		S. D.	.88	1.54	1.06	1.60	1.30		
	Plasma/Saliva Correlation		.82***	.87***	.86***	.95***	.88***		
TESTOSTERONE	Plasma	Mean[a]	5.37	5.54	6.58†	6.73†	6.05	5.40	.014
		S. D.	1.74	1.58	2.60	2.21	2.11		
	Saliva	Mean[a]	.079	.086	.105	.115†	.096	4.19	.030
		S. D.	.033	.019	.030	.036	.033		
	Plasma/Saliva Correlation		.66**	.24	.48*	.37	.51*		

[a] All concentrations reported in ng/ml
**p < .05, one-tailed*
***p < .01, one-tailed*
****p < .001, one-tailed*
†Significantly different (p < .05) from baseline and pre-exercise values

Paired plasma and saliva cortisol concentrations were highly correlated ($r \geq .82$, Table 2). However, plasma:saliva testosterone correlations were consistenly lower ($r \leq .65$, Table 2).

Salivary cortisol concentrations ranged between 2.14 and 2.36 percent of the corresponding plasma concentrations. Corresponding values for testosterone were 1.48 to 1.79 percent. In both cases, there was a nonsignificant trend toward lower ratios during the later sample collections. Salivary hormone concentrations were not correlated with saliva flow rate at any point.

Discussion

Moderately heavy exercise did not affect the relationship between plasma and salivary cortisol. The correlations were consistently high. Mean levels of cortisol did not vary, possibly due to the only moderate exercise intensity (3). Whether this will hold true during heavier or more prolonged exercise is yet to be determined.

The testosterone findings were equivocal. Paired saliva and plasma values were not highly correlated. Changes in mean testosterone levels during exercise were significant and similar in pattern for both plasma and saliva. The low correlation between the paired samples is at odds with previous findings in resting subjects (13), but even the baseline correlation in this study was only $r=.65$. One aspect of our data that might account for this discrepancy was a relatively restricted range of testosterone values compared to those in other studies. Wang, et al. (13) reported a high correlation ($r=.94$) and presented a scatterplot for paired saliva and plasma testosterone values. The scatterplot suggests that elimination of a few extreme samples would have produced results quite similar to ours. Within a restricted range of values, the well-documented individual differences in the ratio of free to total testosterone (24-26) could have had more influence on the saliva-plasma associations. This issue may be important for salivary steroid research in general because similar distributional factors appear to affect some of the reported associations for cortisol (16), 17-hydroxyprogresterone (16), and aldosterone (17). Previous reports of high salivary-plasma or salivary-serum correlations therefore may have depended on the inclusion of samples from persons with endocrine problems or who received drugs that altered steroid levels.

The present findings do not rule out the possibility that salivary testosterone might have been highly correlated with the free plasma testosterone fraction in our subjects. It is commonly held that salivary steroids reflect free plasma steroids more than they do total plasma steroids (6-8,10-13). Unfortunately, this belief is based largely on indirect evidence (e.g., absence of steroid binding globulins in saliva, low salivary steroid concentrations, and similar half-lives of free plasma and salivary steroids). Katz and Shannon (9) have shown that salivary corticosteroids were more highly correlated with free plasma corticosteroids ($r = +.83$) than with total plasma corticosteroids ($r = +.75$), but this difference is too small for final conclusions, even with circumstantial support from other lines of investigation. The fact that total plasma values and salivary values can be relatively independent at times, as in the case of our testosterone data, makes it critical to examine this issue in more detail. Unfortunately, the importance of the free vs. total blood hormone distinction was not anticipated at the outset of this project because of the consistently high correlations reported in other research; therefore, free hormone measures are not available in our data. Despite the expense and difficulty of obtaining both free and total hormone values, further investigation of the differences between the two is essential. This is particularly true if salivary measures are to be employed in studies involving normal populations.

The fact that testosterone levels increased with exercise adds additional data to support the position that testosterone concentrations increase during exercise; previous evidence on this issue has been mixed (2-4).

The main conclusion from our study is that salivary cortisol can be substituted for plasma cortisol in research involving moderately heavy exercise. The equivocal testosterone findings point to a need for direct investigation of

the relationship between salivary steroids and both total and free plasma steroids; this need applies to other steroid hormones as well.

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Saliva may provide a useful alternative to blood for measuring steroid hormones. Total plasma and salivary concentrations of cortisol and testosterone were compared in samples taken twice at rest and twice during exercise to determine whether physical activity level affects the relationship between the two. Correlations were consistently high ($r > .82$) for cortisol, but relatively low for testosterone ($r < .66$). Exercise did not affect either correlation. Salivary cortisol is a reasonable alternative to plasma cortisol

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20. Abstract (continued)

even during exercise. The testosterone results were equivocal as salivary testosterone could be highly correlated with free plasma testosterone despite the low correlation to total plasma testosterone. Closer examination of the free/total plasma hormone distinction was not possible in the present study, but should be an important focus for further research on salivary steroids.

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